SPECIFICITY AND HOST-RELATIONS IN THE TREMATODE GENUS ZOÖGONUS [1]

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The genus *Zoögonus* was erected by Looss (1901) to contain *Z. mirus*, (as type), and *Distomum viviparum* Olsson, 1868. *Zoögonus mirus* was described from two specimens found in the intestine of *Labrus merula* at Trieste. The worms measured 1.55 mm. in length and about 0.45 mm. in width. The following year, Odhner (1902) designated *Zoögonus viviparus* (Olsson, 1868) Looss, 1901 as type of a new genus *Zoögonoides*. In this paper he redescribed *Distomum rubellum* Olsson, 1868, and transferred the species to *Zoögonus*. Like Olsson, he found the parasites in *Labrus berggylta* (syn. *L. maculatus*) from the west coast of Sweden, but the examination of twenty fishes at the Zoölogical Station of Kristineberg disclosed only two infections and only a few worms were obtained. The specimens were yellowish in color, 1.1–1.4 mm. in length and 0.45 mm. in greatest width. *Zoögonus rubellus* was distinguished from *Z. mirus* on the size of suckers and length of the miracidia. Although Looss and Odhner were two of the ablest students of the trematodes, their observations on *Zoögonus* were limited to the study of very few specimens.

The specific identity of *Z. mirus*, questioned by competent investigators, still remains an unsolved problem. Goldschmidt (1902, 1905), after comparing specimens collected at Trieste with the descriptions of *Z. mirus* and *Z. rubellus*, stated that there were no morphological differences. The dimensions of suckers and miracidia, characters used by Odhner to separate the species, were found to be variable and hence invalid as specific criteria. Furthermore, Goldschmidt was unwilling to differentiate the species on the presence or absence of yellow pigment in the tissues. Nicoll (1909), who reported *Z. rubellus* as consistently abundant in *Anarhichas lupus* from St. Andrews Bay, described the worms as pale yellow in color, 0.75–1 mm. in length and about one-half

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as wide as long. Referring to the descriptions of Looss, Odhner and Goldschmidt, Nicoll stated, "My specimens agree best with Goldschmidt's description." It is significant that the worms studied by Nicoll and Odhner came from the same region, whereas Goldschmidt's material was collected in the Mediterranean and presumably was identical with that of Looss. Concerning specific determination, Nicoll expressed the opinion that, "Looss's *Zoögonus mirus* is in all probability identical with Odhner's *Z. rubellus* . . . at any rate, features sufficient to distinguish them are not at present apparent."

In a systematic review of the family Zoögonidae, Odhner (1911) maintained that the specimens of *Zoögonus* from the Mediterranean and North Sea are specifically distinct. After collecting material from both locations, he distinguished the two species on differences in size and color, size of suckers and shape of sucker cavities, location of the acetabulum, length of the digestive ceca, position of the cirrus sac, and number of eggs in the uterus. Odhner stated that extended specimens of *Z. mirus* never exceed 0.6 mm. in length and 0.2 mm. in width, whereas similar specimens of *Z. rubellus* measure 0.9–1.2 mm. in length and about 0.25 mm. in width. He noted that the pharynx and the miracidia are approximately the same size in the two species, but appear to be larger in the smaller specimens of *Z. mirus*.

The specimens of *Z. mirus* studied by Odhner were apparently contracted and probably not entirely mature. His statement that extended worms never exceed 0.6 mm. in length cannot be accepted, since the type specimens described by Looss measured 1.55 mm. in length. The number of eggs in the uterus obviously is correlated with the degree of sexual development, and the other features employed by Odhner to differentiate the species manifest so much variation that subsequent authors have disagreed on the identity or distinctness of the European species of *Zoögonus*. It is apparent that morphology of the adult stages is inadequate for a final solution of the problem.

Although a beginning has been made on the life history of *Zoögonus*, information from this source is still too fragmentary to permit final specific determination. In a series of papers, Timon-David (1933, 1934, 1936, 1938) described encysted metacercariae, identified as larvae of *Z. mirus*, in sea-urchins collected in the Gulf of Marseilles and along the coast of Roussillon. The metacercariae were found only in the muscles of the lantern of Aristotle. The degree of infection was variable and from one to sixty cysts were recovered from individual urchins. The incidence of infection in *Paracentrotus lividus* reached 50 to 60 per cent, a somewhat lighter infection was common in *Sphaerechinus granularis*, only a few cysts were recovered from *Arbacia acquituberculata*,

whereas no infection was observed in *Echinus acutus* or *Psammechinus microtuberculatus*. All the parasites apparently belonged to a single species. The cysts increased in size with the development of the metacercariae, and measured from 0.15 to 0.25 mm. in diameter. A cyst (1934, Fig. 1), fixed in picro-formol-alcohol solution under moderate compression, measured about 0.4 mm. in diameter, according to the scale accompanying the drawing. The cyst wall measured 0.003 mm. in thickness and was not surrounded by a connective tissue capsule. The worm was bent upon itself, with the dorsal surface applied to the wall of the cyst. Released from their cysts, the mature larvae averaged 0.6 mm. in length and 0.2 mm. in width. Specimens from *P. lividus, A. aequituberculata* and *S. granularis* (1934, Figs. 2, 3, 4), fixed in extended condition, measured 0.83, 0.93 and 0.66 mm. respectively (length calculated from scales accompanying the figures). Timon-David (1936) reported that metacercariae fed to *Blennius gattoruginae* excysted and persisted in the intestine for 45 days. Such a specimen, figured in the report, measured 0.64 mm. in length and was little if any farther advanced in development than larvae freshly removed from their cysts. The observation of Timon-David, that remains of sea-urchins were frequently present in the intestine of *Labrus merula,* supports his opinion that the metacercariae from the urchins are actually larval stages of *Z. mirus*. In his (1934) paper he recalled that the development of the miracidia has been known since the accounts by Looss (1901), Goldschmidt (1902) and Wassermann (1913), but that the first intermediate host remains as yet unknown. He expressed the belief that the cercarial stages are to be sought among the gastropods of the region.

In a report on larval trematodes from the region about Roscoff, Finistère, Stunkard (1932) described a tailless cercaria, *C. reticulatum* from *Nassa reticulata*, which shows such remarkable morphological agreement with the metacercariae described by Timon-David that the two must be closely related and may possibly belong to the same species. One item in the description of Stunkard requires correction. In the figure, the pharynx is represented as only a short distance in front of the acetabulum, whereas notes made at the time state that the pharynx is situated about midway between the suckers.

A single species of *Zoögonus* has been recorded from the Atlantic coast of North America. It was first described in the cercarial stage by Leidy (1891), who named it *Distomum lasium*. The larvae develop in *Nassa obsoleta*. Subsequent studies on the cercaria were reviewed by Stunkard (1938), who completed the life cycle. The cercariae encyst in polychaete annelids, principally *Nereis virens*. Natural infections were found in eels and sexually mature specimens were recovered after ex-

perimental infection of the eel and toadfish. Comparison of adult specimens with descriptions of *Z. rubellus* and *Z. mirus* provided no positive basis for specific distinction and so notwithstanding certain differences in hosts, life cycle, and morphological details, Stunkard regarded the American specimens as specifically identical with *Z. rubellus* and *Z. mirus*.

Subsequent studies of the European species carried on at the Station Zoologique de Wimereux in 1939 and of the American species at the Marine Biological Laboratory in Woods Hole during 1940, have yielded such discordant results that the question of specific identity must be reconsidered. The results of these observations were reported in abstract (Stunkard, 1940).

A sojourn at Wimereux, France, from July 8 to August 14, 1939, provided an opportunity to reexamine European phases of the *Zoögonus* problem. Metacercariae were found in *Psammechinus miliaris*, the common sea-urchin of the region. Urchins were collected at different locations from the Port de Boulogne to Ambleteuse, a stretch of some ten kilometers. The heaviest infection appeared in specimens from the Port de Boulogne where encysted larvae were recovered from more than 50 per cent of the urchins dissected. Different individuals harbored from one to thirty-six metacercariae. Lighter incidence and degree of infection were found in urchins taken between Boulogne and Ambleteuse. All of the larvae appeared to belong to a single species.

The metacercariae were encysted in the muscles and connective tissue of the lantern of Aristotle. The cysts were transparent, with no obvious reaction of the host to the parasite. The cyst wall was thin, colorless and very tough. The cysts measured from 0.2 to 0.28 mm. in diameter. The larvae, freed from their cysts, measured 0.42 to 0.65 mm. in length and 0.15 to 0.22 mm. in width. The cuticula was spined, although the spines were reduced in size and number behind the level of the testes. Larvae fixed under cover-glass pressure measure up to 0.95 mm. in length and a small one (Fig. 3), apparently equally mature but fixed without pressure, is only 0.37 mm. in length. When the worms are fixed without compression, the preacetabular portion bends ventrad, so that in mounted specimens the oral sucker may appear above or below and immediately in front of the acetabulum (Figs. 1, 2, 3). A representative specimen, fixed under slight pressure and shown in Fig. 4 is 0.75 mm. in length. The acetabulum, situated near the middle of the body, measures 0.08 by 0.086 mm. in diameter. The pharynx is located about midway between the suckers and measures approximately 0.06 mm. in diameter. When the specimen was extended the pharynx tended to be longer than wide. Its anterior end is dentate and the nuclei of the organ are concentrated

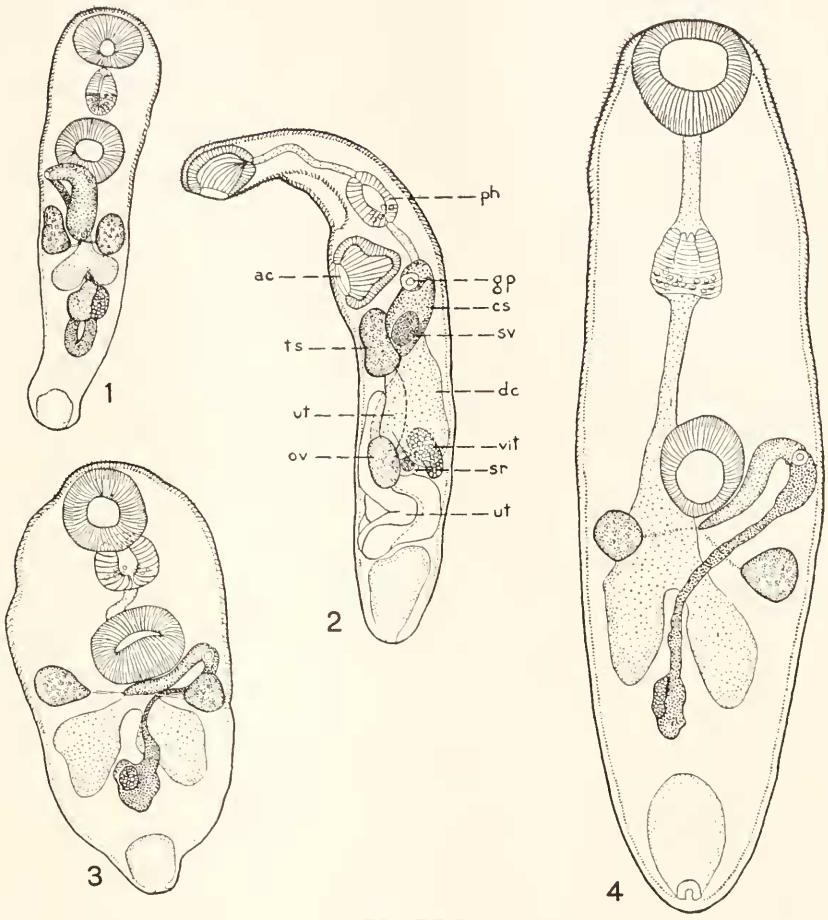

PLATE I

Abbreviations

ac	acetabulum	*sr*	seminal receptacle
cs	cirrus sac	*sv*	seminal vesicle
dc	digestive cecum	*ts*	testis
gp	genital pore	*ut*	uterus
ov	ovary	*vit*	vitellaria
ph	pharynx		

All figures are drawn to the same scale from fixed, stained and mounted specimens.

Fig. 1. Mature metacercaria, developed six weeks in *Nereis virens*, fed to a toadfish and removed two days later; Woods Hole. One of the largest specimens, fixed without compression, anterior end bent ventrad; dorsal view.

Fig. 2. Sexually immature specimen, developed six weeks in *N. virens* and six weeks in an eel; Woods Hole. Shows preacetabular ventral bending of body in specimens fixed without compression; lateral view from left side.

Fig. 3. Mature metacercaria from *Psammechinus miliaris;* Wimereux. One of the smallest specimens, fixed without compression, anterior end bent ventrad; ventral view.

Fig. 4. Mature metacercaria from *P. miliaris;* Wimereux. An average size specimen, fixed under cover-glass pressure, 0.75 mm. long; ventral view.

in its posterior half. The excretory system was worked out completely and agrees in detail with that of *Cercariacum reticulatum* and with that of the American species of *Zoögonus*.

Measurements of the metacercariae from Wimereux do not differ greatly from those given by Timon-David for metacercariae from sea-urchins in the Mediterranean. The suckers in my specimens are slightly smaller than those measured by Timon-David, although his figures show the apertures of the suckers to be wide open and the specimens may have been more flattened. He figured the acetabulum slightly behind the middle of the body, whereas in my material it tends to lie slightly in front of the middle, although this feature is variable and changes with the extension or retraction of anterior and posterior regions of the body. Consequently, it appears likely that the specimens from Wimereux are specifically identical with those from the Mediterranean.

After discovery of the metacercaria, attempts were made to find the other stages in the life cycle of the European species of *Zoögonus*. Examination of many fishes, including several specimens of *Labrus sp.*, were fruitless. In view of the possibility, expressed previously, that the tailless larva, *C. reticulatum*, may represent a stage in the life history, wide search was made for it. The host, *Nassa reticulata*, is abundant in the region but the examination of more than 1200 specimens did not yield a single infection with tailless cercariae. Over 800 of these snails were collected from mud between rocks of the breakwater in the Port de Boulogne, and sea-urchins taken from these rocks were heavily infected with metacercariae. Other mollusks examined for *Zoögonus* larvae, with negative results, included 240 *Mytilus edulis*, 146 *Barnea candida*, 28 *Tapes pullastra*, 10 *Ensis sp.*, 240 *Patella vulgaris*, 35 *Purpura lapillus*, 34 *Littorina obtusa*, 86 *L. rudis*, 56 *L. littorea*, and 45 *Gibbula cineraria*.

To determine whether annelids as well as echinoderms harbor metacercariae of the European species of *Zoögonus*, worms were carefully dissected under a binocular microscope. The examination of 14 *Eunereis longissima*, 12 *Nereis errorata* and representatives of other unidentified polychaetes from the Port de Boulogne did not disclose any metacercariae.

In view of the failure to discover other stages in the life cycle of *Zoögonus* in the Wimereux area, the origin of the infection in the sea-urchins and subsequent fate of the larvae are entirely problematical. The completion of the life history by trematode parasites would be difficult in this region since the tides have an amplitude of eight to ten meters and the collecting grounds, exposed at low tide, are covered by an enormous volume of water six hours later.

Comparison of the larval stages of *Zoögonus* found along the north coast of Europe and the eastern coast of North America shows slight but apparently significant differences. Cercariae from *Nassa reticulata* at Roscoff average slightly larger than those from *N. obsoleta* at Woods Hole and the suckers are also larger. In the European form the ranges of size are: acetabulum, 0.068–0.076; oral sucker, 0.076–0.085; and pharynx, 0.03–0.04 mm., whereas measurements of the corresponding structures in the American form are: 0.062–0.075; 0.043–0.055; and 0.022–0.028 mm. Moreover, in the European form the prepharynx is relatively shorter and the pharynx is about midway between the suckers, whereas in the American form the pharynx is farther posteriad and frequently overlaps the acetabulum. The metacercariae from Wimereux and from Woods Hole show the same differences as the cercariae from the two regions. Cysts of the European form are slightly larger, the metacercariae are larger (compare Figs. 1, 2, and 3) and the relative sizes of suckers persists. Average measurements of ten specimens from the two localities give the following sizes (dimensions of Wimereux specimens first, of Woods Hole specimens second): acetabulum 0.085 *vs.* 0.075; oral sucker 0.09 *vs.* 0.065; pharynx, 0.06 *vs.* 0.042 mm.

To determine whether the American species of *Zoögonus* may occur in sea-urchins as well as polychaete annelids, attempts were made at Woods Hole in the summer of 1940 to infect urchins with *Distomum lasium* (= *C. lintoni*). Many freshly dredged urchins, both *Arbacia punctulata* and *Strongylocentrotus drobachiensis*, were dissected with negative results. Since enormous numbers of these animals have been used during the past forty years for embryological and other studies without the reported finding of metacercariae, natural infection with trematode larvae must be absent or very slight. Portions of dissected urchins, including the denticles and attached tissues, were placed in dishes of sea water with scores of naturally emerged cercariae of *Zoögonus*. The larvae crawled about over the tissues but did not penetrate or encyst. They were not attracted toward intact sea-urchins or dissected portions of them. Single urchins were exposed for several hours during the day in finger bowls to hundreds of cercariae and maintained during the intervening time in large aquaria. Dissection of the urchins later did not disclose any metacercariae.

Although the factors concerned with infection of the secondary intermediate host are virtually unknown, it is apparent that experiments devised to secure experimental infection in the laboratory must approximate natural conditions as closely as possible. Accordingly, on August 16, 1940, twenty specimens of *N. obsoleta* from which cercariae were emerging in large numbers were placed in each of two aquaria. Fifteen speci-

mens of *A. punctulata* were added to one aquarium; five specimens of *A. punctulata* and five specimens of *S. drobachiensis* to the other. After an interval of a week, dissection of the urchins was begun. No infection was found in *S. drobachiensis* but *Zoögonus* larvae were recovered from eleven specimens of *A. punctulata*. These urchins, examined in the period from August 23 to September 11, yielded 79 cysts in which the larvae were dead, 60 cysts containing living larvae, 32 unencysted, dead larvae, and 16 unencysted, living larvae. The dead, encysted larvae were often partly disintegrated. Live larvae in cysts had extruded their stylets, but showed no evidence of development. The gonads were no larger than those of the cercaria and the ducts of the penetration glands were still visible. It is evident from these results that cercariae of the American species of *Zoögonus* will enter and encyst in sea-urchins and that they may live there for a time. But no development was observed and the finding of so many dead larvae, both free and encysted, indicates that *A. punctulata* is not a suitable host. It is probable, therefore, that these sea-urchins are not involved in the life cycle of the parasite.

The problem of specificity in host-parasite relations can be solved only by the experimental methods developed in studies on the life cycles of parasites. Formerly it was believed that different species of hosts harbored different parasites. In the case of trematodes, it is now known that a single parasitic species may infect a wide variety of hosts. *Allassostoma parvum* may infect frogs and turtles; *Zygocotyle lunata* may infect birds, rodents and ruminants; *Cryptocotyle lingua* may infect birds, rodents and carnivores; *Notocotylus urbanensis* may infect ducks and muskrats; *Fasciola hepatica* may infect cattle, pigs, rodents, the elephant, kangaroo and man; *Echinostoma revolutum* may infect various species of birds and mammals; *Psilostomum ondatrae* may infect the muskrat, duck, pigeon and canary. These examples, selected from a large list, represent five families and show that the possibility of multiple hosts is general. Furthermore, as a result of development in widely separated hosts, representatives of a single trematode species may manifest morphological differences which under other conditions might reasonably be regarded as specific. Specimens of *F. hepatica* from a guinea pig and others from a cow would hardly be assigned to the same species on the basis of morphology.

The digenetic trematodes manifest a comparable lack of specificity in their intermediate hosts. This is true particularly in cases involving a second intermediate host, often nothing more than a "transfer host" in which no development occurs. The condition is similar to that in *Fasciola* and *Zygocotyle*, where cercariae encyst on vegetation or other objects which are eaten by the final host. Even in the first intermediate

host, specificity may be far from rigid. For *Fasciola hepatica,* which has become cosmopolitan in distribution, snails belonging to the following genera may serve as first intermediate hosts: *Lymnaea, Galba, Bulinus, Physopsis, Physa, Stagnicola, Fossaria, Pseudosuccinea* and *Ampullaria.* In any particular region, one variety of snail is selected, but in different regions the species is different.

The data on *Zoögonus* are hard to interpret. In view of the lack of specificity in the life cycles of other trematodes, it is not impossible that a single species of *Zoögonus* employs different primary, secondary and definitive hosts on the two sides of the Atlantic ocean. In such event, the morphological variations are readily explained. On the other hand, the bionomic and morphological differences may represent valid specific criteria. This opinion is supported by cytological observations. According to Goldschmidt (1905), *Z. mirus* has 10 chromosomes, while Brooks (1930) found 12 chromosomes in the American form. At present there is no basis for a positive distinction between species of *Zoögonus* from the North Sea and the Mediterranean, but it appears probable that the European and American forms are specifically distinct. If this proves to be true, the American species is *Z. lasius* (Leidy, 1891) Stunkard, 1940.

Summary

Encysted metacercariae of *Zoögonus* are reported from the sea-urchin, *Psammechinus miliaris,* at Wimereux, France. Comparison with descriptions of other larval stages found at Roscoff and Marseilles indicates that all belong to the same species. Attempts to infect sea-urchins at Woods Hole with the American form of *Zoögonus* were only partially successful. Bionomic and morphological differences between the European and American representatives of *Zoögonus* are discussed. It appears probable that they belong to different species.

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